Mineralogical Constraints on the Biochemistry of Bacteria Attached to Basalt

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INTRODUCTION

Recent literature suggests that >95% of the microorganisms inhabiting the subsurface are attached to the geologic substratum. Such attachment may result in genetically controlled and environmentally influenced physiological changes within the bacterial cell. Microorganisms colonizing geologic materials have been characterized with a variety of methods; however little information exists regarding the in situ biochemistry of bacterial cells attached to various mineral phases within a heterogeneous substratum. In this study, we investigated the variation in spectral markers associated with a pure culture of bacteria in contact with various mineral phases using synchrotron radiation-based Fourier transform infrared microspectroscopy (SR-FTIR). Petrographic thin sections of olivine basalt and the four individual major mineral phases within the basalt were spectrally characterized before and after exposure to a growth culture of *Burkholderia cepacia* G4. The bacteria-mineral interface spectra were collected using the 1.4.3 Beamline experimental endstation at the Lawrence Berkeley National Laboratory (LBNL) Advanced Light Source (ALS). Absorption spectra were recorded in the 4000-650 cm^{-1} infrared region at a spectral resolution of 4 cm^{-1} and microscope-focused spot size of 10 microns.

Two experimental designs were used in this study. In the first experiment, *Burkholderia cepacia* G4 cells were cultured overnight, centrifuged and washed then resuspended in phosphate buffer. The concentrated cells were pipetted onto the surface of sterilized 1-mm thick slices of plagioclase, olivine, augite, and ilmenite (the four major minerals found within olivine basalt). After approximately 40 minutes contact time, spectra were collected of the cells on the mineral specimens. In the second experiment, *B. cepacia* G4 cells were removed from stock agar plates and suspended in phosphate buffer. The cells were then added to microcosms which contained a minimal growth media and the four individual mineral specimens, basalt specimens, and a composite microcosm containing all four mineral specimens (plagioclase, olivine, augite, ilmenite). The microcosms were placed on a slow rotary shaker and left at room temperature for 17 days. Growth solution was removed every day and replaced with new growth solution to prevent the build-up of waste products. At the end of the 17 days, the mineral specimens were taken from the microcosms and gently rinsed three times with phosphate buffer to remove any unattached cells. The cultured mineral specimens were then placed sterile petri dishes on sterile filters saturated with sterile de-ionized. The petri dishes were then stored in a

glass chamber at 27°C and 100% relative humidity until spectra were collected with the SR-FTIR.

Results from the overnight cultures show significant variation in spectra obtained from the same pure culture when in contact with different minerals. The spectra shown in Figure 1 were collected approximately 40 minutes after the bacterial cells were pipetted onto the mineral surface. The background spectra of the individual minerals have been subtracted, revealing the spectral markers associated with the bacterial cells.

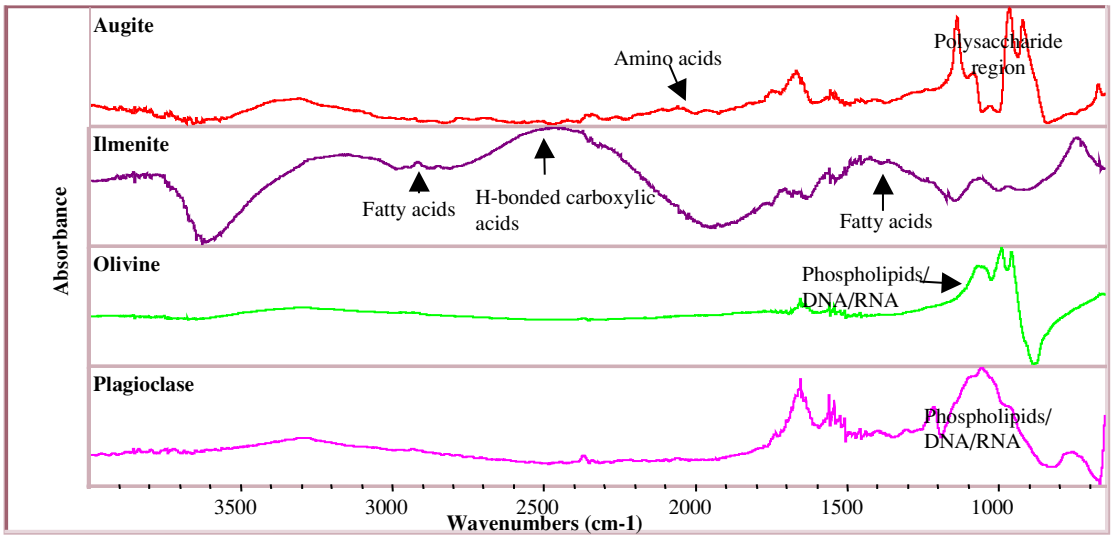

Figure 1

All four spectra have the bands assigned to the protein Amide I and Amide II peaks at 1650 cm^{-1} and 1550 cm^{-1}, respectively, which indicate the presence of bacterial cells. However, variations are evident in the presence of various functional groups that can be correlated to biochemical compounds.

Spectra taken of the individual microcosm samples indicate moderate, unevenly distributed colonization on plagioclase and basalt, minimal colonization of olivine and augite, and no colonization of ilmenite. Spectra collected from the composite microcosm minerals showed no presence of bacteria on any of the four minerals. However, the basalt samples, which contain the same four minerals in a natural matrix, did have strong spectral markers indicating bacterial attachment and presence of various biochemical compounds (Fig. 2). Additionally, mapping of the basalt samples revealed preferential bacterial attachment to plagioclase (Fig. 3).

CONCLUSIONS

Investigating bacterial attachment using individual or composite mineral microcosms does not simulate the natural basalt matrix. The pure culture used in this study grew very well on basalt, with much lower concentrations of an additional carbon source, than in the presence of the same minerals as individual microcosms or as composite microcosms.

Under these experimental conditions, *B. cepacia* G4 exhibits preferential attachment to plagioclase within the basalt matrix.

SR-FTIR is valuable tool in assessing the *in-situ* biochemistry of bacterial attachment to geologic materials.

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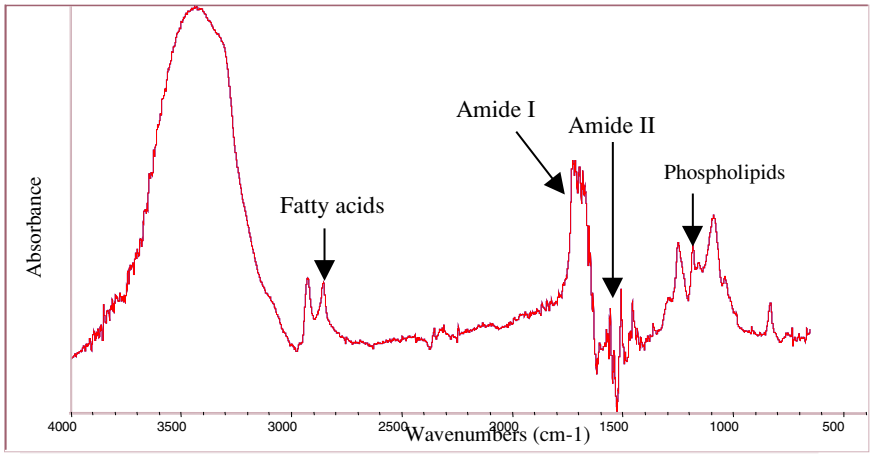

Figure 2

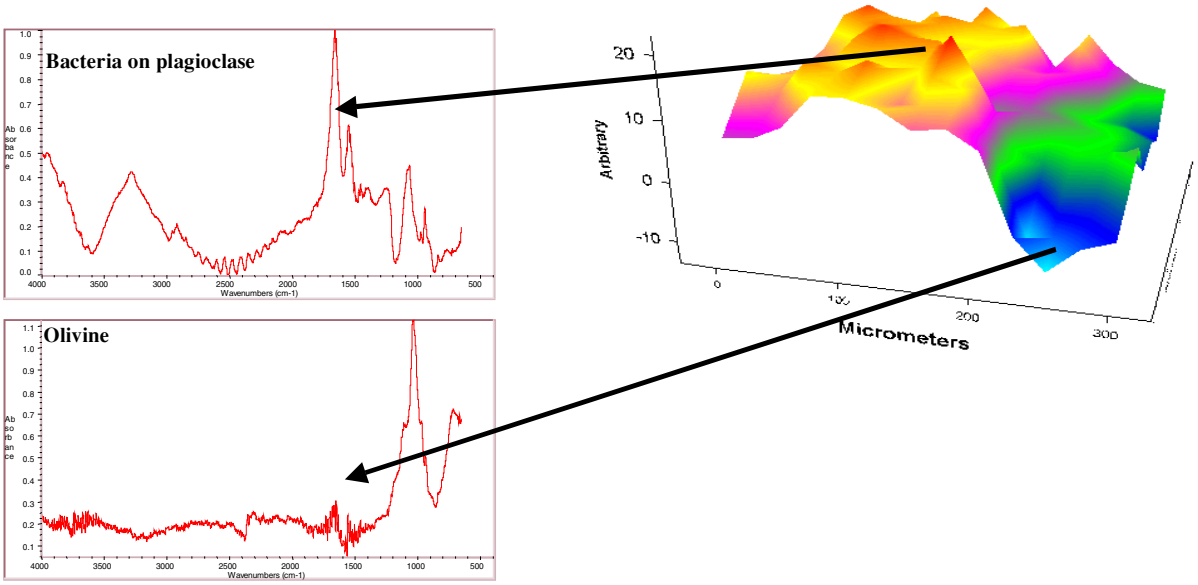

Figure 3. Area map of a microcosm basalt sample showing the presence of bacteria on plagioclase and the absence of bacteria on olivine based upon the presence of the Amide I band at 1650 cm^{-1}.